

THE AMENDMENTS

In The Specification:

Please amend the paragraph starting at page 23, line 17:

For rehydration, the spray-fixed smears are incubated in fresh 50% EtOH on a rocking device. The PEG film produced by the fixation procedure is removed by intensive rinsing. Then, the smears are rinsed in Aqua bidest. The smears are incubated with proteinase K (10µg/ml in PBS) for 10 min at 37°C. Then, the slides are transferred to washing buffer (PBS / 0.1% ~~Tween~~²⁰ TWEEN[®] 20) and finally, the area containing the cells is surrounded with a lipid-pencil.

Please amend the paragraph starting at page 26, line 20:

The tissue sections are rehydrated through incubation in xylene and graded ethanol, and then transferred to Aqua bidest. Conventional smears and liquid-based cytological samples (THINPREP[®]) are rehydrated in ethanol (50%) for 10min, and transferred in Aqua bidest. Antigen retrieval is carried out with 10mM citrate buffer (pH 6.0) for p16^{INK4a}, Ki67, and PCNA. Therefore, the slides are heated in a waterbath for 40 min at 95 - 98°C. The slides are cooled down to RT for 20 minutes, transferred to washing buffer (50mM Tris-HCl, 150mM NaCl, 0.05% ~~Tween~~²⁰ TWEEN[®] 20 / DakoCytomation: code no.: S3006), and finally, the samples are surrounded with a lipid-pencil.

Please amend the paragraph starting at page 27, line 1:

The slides are then incubated with the primary antibodies, mouse anti-human p16^{INK4a} antibody (3.48 µg/ml), rabbit anti-Ki67, and rabbit anti-PCNA (each 1:25) for 30 min at RT. The slides are then rinsed with washing buffer and placed in a fresh buffer bath for 5 min. Excess buffer is tapped off, and the specimen is covered with 200µl of the secondary reagent (containing goat anti-mouse antibody, ~~AlexaFluor[®]~~ ALEXA FLUOR[®] 488 conjugated and goat anti rabbit antibody and, ~~Alexa-Fluor[®]~~ ALEXA FLUOR[®] 546 conjugated) and then incubated for 30min at RT. Then slides are washed two times as before and directly mounted with a special mounting medium for fluorescence.

Please amend the paragraph starting at page 28, line 20:

Example 6: Immunoenzymatic detection of the over-expression of p16^{INK4a} and Ki67 in histological samples of the uterine cervix (sequential double staining)

Sections of formalin-fixed, paraffin-embedded tissue samples of the cervix uteri were immunoenzymatically double-stained using antibodies specific for p16^{INK4a} and Ki67.

Please amend the paragraph starting at page 28, line 24:

The tissue sections were rehydrated through incubation in xylene and graded ethanol, and transferred to Aqua bidest. Antigen retrieval was carried out with 10mM citrate buffer (pH 6.0) for p16^{INK4a} and Ki67. Therefore, the slides were heated in a waterbath for 40 min at 95 – 98°C. The slides were cooled down to RT for 20 minutes and transferred to washing buffer (DakoCytomation).

Please amend the paragraph starting at page 28, line 28:

Endogenous peroxidase activities were blocked with 3% [[H₂O₂]] H₂O₂ (DakoCytomation) for 5min at RT.

Please amend the paragraph starting at page 29, line 1:

After washing the slides for 5min at RT, they were incubated with the first primary antibody, mouse anti-human p16^{INK4a} antibody (MTM) for 30 min at RT, and were then rinsed with washing buffer and placed in a fresh buffer bath for 5 min. Excess buffer was tapped off and each specimen was covered with 200µl of the secondary reagent (EnVision goat anti mouse – Peroxidase/ DakoCytomation), and incubated for 30min at RT. Then slides were washed three times as before. For the chromogenic visualization, DAB (DakoCytomation) was used by incubating the slides with the substrate chromogen complex for 10min at RT. The reaction was stopped in deionized water, and the slides placed in wash buffer.

Please amend the paragraph starting at page 29, line 19:

The microscopic examination of the slides revealed that cells immunoreactive with p16^{INK4a} and Ki67 were found only in samples that were identified microscopically as samples of

dysplastic lesions. Cells stained by the ~~p16INK4a~~ ~~p16~~^{INK4a} -specific reaction, originating from metaplasias, were not stained by the reaction specific for Ki67. Microscopic inspection of the cell proliferation marker staining showed that metaplastic cells over-expressing ~~p16INK4a~~ ~~p16~~^{INK4a} were not immunoreactive with antibodies directed against Ki67. Samples containing dysplastic tissue areas, in contrast, comprised cells that were immunoreactive with Ki67 and with antibodies directed against ~~p16INK4a~~ ~~p16~~^{INK4a}. So, in contrast to dysplasias, in metaplasias, no cells were double-stained using the Ki67 and ~~p16INK4a~~ ~~p16~~^{INK4a} specific antibodies.

Please amend the paragraph starting at page 30, line 3:

~~Merckofix~~[®] MERCKOFIX[®] fixed cytological samples (conventional smears and liquid-based cytology (~~ThinPreps~~[®]) (THINPREP[®])) of the cervix uteri were immunoenzymatically double-stained using antibodies specific for p16^{INK4a} and Ki67.

Please amend the paragraph starting at page 30, line 10:

Endogenous peroxidase activities were blocked with 3% [[H₂O₂]] H₂O₂ for 5min at RT.